How to halve ploidy - Lessons from budding yeast meiosis

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Running title- Halving ploidy during yeast meiosis

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Abstract

Maintenance of ploidy in sexually reproducing organisms requires a specialized form of cell division called as meiosis that generates genetically diverse haploid gametes from diploid germ cells. Meiotic cells halve ploidy by undergoing two rounds of nuclear division (meiosis I and II) after a single round of DNA replication. Research in *Saccharomyces cerevisiae* (budding yeast) has shown that four major deviations from mitotic cell cycle during meiosis are essential for halving of ploidy. The deviations are a) formation of a link between homologous chromosomes by crossover b) monopolar attachment of sister kinetochores during meiosis I c) protection of centromeric cohesion during meiosis I d) suppression of DNA replication following exit from meiosis I. In this review we present the current understanding of the above 4 processes in budding yeast and examine the possible conservation of molecular mechanisms from yeast to humans.

Key words- meiosis, gametogenesis, ploidy, cell cycle, shugoshin, monopolin, FEAR

Abbreviations APC- Anaphase Promoting Complex Cdk- Cyclin-dependent kinase ; DSB- Double-strand break ; FEAR -Cdc Fourteen Early Anaphase Release; MT- microtubule ;

Introduction

Sex entails mixing of paternal and maternal genomes which allows union of two or more favorable mutations of different genotypes into a single genotype in the progeny. This is in contrast to the less plausible scenario in asexually reproducing organisms where favorable mutations need to occur in the same cell lineage. Sex therefore enables rapid evolution of a population in comparison to an otherwise equivalent population that reproduces asexually. However the requirement of two parents in the sexual mode of reproduction introduces a problem about maintenance of ploidy (chromosome number). The ploidy of a sexually-reproducing species would double every generation if diploid (somatic) cells were used as gametes. In order to maintain ploidy, sexually reproducing organisms have evolved a specialized way of cell division called meiosis that produces haploid gametes from diploid germ cells.

How do meiotic cells halve their ploidy? Meiotic cells undergo two nuclear divisions after one round of DNA replication which is in contrast to somatic cells where ordinarily a round of nuclear division is followed by a round of DNA replication. Research on meiosis in various model systems like *Saccharomyces cerevisiae* (budding yeast), *Schizosaccharomyces pombe* (fission yeast), *Drosophila melanogaster* (flies), *Caenorhabditis elegans* (worms) and *Mus musculus* (mice) have given valuable insights into how this might be accomplished. In this review we will discuss how budding yeast research has led to understanding of meiotic cell cycle events like entry into meiosis, DNA recombination, protection of centromeric cohesion, monopolar attachment of sister kinetochores and exit from meiosis I. At the end we will discuss whether the above mechanisms are conserved in higher eukaryotes. An understanding of meiosis is relevant to human health as errors in regulation of meiotic cell cycle could result in fetal aneuploidy and spontaneous abortions in humans [1].

Overview of mitotic cell cycle in budding yeast.

To fully appreciate the logic of meiosis, it is necessary to review how the mitotic cell cycle works as several elements of the mitotic cell cycle machinery are used during meiosis either with no changes or with subtle improvisations. Cell cycle can be divided into G1, S, G2 and M phases. Cells replicate their DNA during S (synthesis)-phase and segregate chromosomes and divide their cytoplasm (cytokinesis) during M (mitotic)-phase. These phases are

separated by two gaps namely, G1 and G2 phases. Progression through the cell cycle is regulated by the highly conserved family of proteins called as cyclin-dependent kinases (Cdk). The kinase activity of Cdk on its own is very little but is stimulated by binding of Cdk to its regulatory subunit called as cyclin. Budding yeast has a single Cdk which is encoded by the *CDC28* gene. Cdc28 associates with distinct cyclins during different phases of the cell cycle and controls the activity of downstream effector proteins by phosphorylation to orchestrate cell cycle transitions. Entry into S-phase is promoted by Cdk bound to cyclins Cln1 and Cln2. Cdk bound to cyclins Clb5 and Clb6 are involved in DNA replication. Cdk bound to cyclins Clbs1-4 regulates progression through mitosis. Oscillating levels of cyclins leads to periodic activation of Cdk which ensures the correct sequence of events like budding, DNA replication, chromosome segregation and cytokinesis during the cell cycle. When errors occur during the cell cycle, control mechanisms referred to as checkpoints delay or prevent cell cycle progression until the previous step has been successfully completed.

Cell cycle is coordinated with chromosome segregation. Replication of DNA during S-phase generates sister chromatids which are held together by a highly conserved protein complex called as cohesin. Cohesin is composed of 4 subunits Smc1, Smc3, Scc1 and Scc3 and forms a 50nm ring that holds sister chromatids together by trapping them topologically within it [2-4]. The spindle pole bodies (functional equivalent of centrosomes in mammals) duplicate during G1/ S phase. As cells enter mitosis, the spindle pole bodies separate and a bipolar spindle is formed. A specialized structure composed of several multi-protein sub-complexes called as the kinetochore is assembled at the centromere on every chromosome. The kinetochore has the ability to bind to growing ends of microtubules. When sister kinetochores bind to microtubules emanating from opposite spindle poles, their pole-ward movement is opposed by sister chromatid cohesion. The ensuing tug-of-war generates tension that stabilizes microtubule- kinetochore connections. When all the sister kinetochores are bi-oriented on the mitotic spindle (metaphase), the spindle checkpoint (a surveillance mechanism that delays cell cycle progression in response to kinetochore-microtubule binding defects) is inactivated. This results in activation of the ubiquitin ligase called as Anaphase Promoting Complex (APC) bound to the substrate specificity factor Cdc20 (APC/Cdc20). APC/Cdc20 activates a cysteine protease separase (Esp1) by targeting its inhibitor securin (Pds1) for proteasomal degradation by ubiquitination. Separase cleaves the cohesin subunit

Scc1 which destroys sister chromatid cohesion and triggers the movement of sister chromatids towards the opposite poles of the cell (anaphase). Following anaphase cells exit from mitosis by abrogating M-Cdk (mitotic Cdk) activity. This is achieved by destruction of Clb's and dephosphorylation of Cdk targets that result in spindle disassembly, cytokinesis (separation of cytoplasm) and preparation for the next round of DNA replication.

Entry into meiosis

Budding yeast cells can exist as either haploids or diploids. Haploid cells could be one of two possible mating types '**a***'* or '*α'* (alpha). The mating type is specified by a single locus called as *MAT* and is determined by whether the *MAT* locus contains the *MAT***a** (encoding gene a1) or the *MATα* allele (encoding genes *α1* and *α2*). When haploid cells of opposite mating type are in close juxtaposition they mate using a pheromone-based signaling pathway. The cytoplasmic fusion of the two cells is followed by fusion of their nuclei resulting in the formation of a diploid cell. When subjected to nutritional starvation, only diploid cells (*MAT* **a**/*MATα)* can enter meiosis which is a key developmental decision in budding yeast. Meiosis is coupled to sporulation as the 4 haploid products are packaged as spores in a sac referred to as the ascus. The fact that it is possible to analyze all the 4 products of an individual meiosis in budding yeast has been invaluable in dissecting the mechanism of meiotic recombination and chromosome segregation.

Yeast cells enter meiosis on the basis of 2 kinds of cues.

a) genetic – Cells have to express both *MAT***a** and *MATα* genes. This ensures that only diploid and not haploid cells enter meiosis.

b) environmental - Absence of glucose and nitrogen and presence of a non-fermentable carbon source in the growth medium. The requirement of a non-fermentable carbon source ensures that only respiration-competent cells can enter meiosis. The coupling of nutrient starvation to sporulation enhances the chances of survival of yeast cells under adverse conditions as the spores are genetically distinct and have the ability to stay dormant for long periods of time.

Integration of these 2 cues occurs at the promoter of the master transcription factor Ime1 (*I*nitiator of *me*iosis) which is required for expression of several early-meiosis genes involved

in entry into meiosis, DNA replication and homologous recombination [5,6]. During vegetative growth multiple mechanisms ensure that *IME1* is not expressed. In haploid cells the Zinc-finger protein Rme1 (Repressor of *me*iosis) binds to *IME1* promoter and prevents its transcription [7]. Rme1 also binds to *CLN2* promoter but this activates *CLN2* expression [8]. Cln2 interferes with entry into meiosis (see below). In diploid cells, the *MATa1 and MATα2* gene products form a complex that represses transcription of *RME1* [7]. In addition, the *MAT a1/MAT α2* complex activates expression of *IME4* a putative RNA methyltransferase which in turn enhances *IME1* expression through an unknown mechanism [9,10].

How does the absence of glucose and nitrogen trigger entry into meiosis? Ime1 is recruited to the promoters upstream of early-meiosis genes and activates their transcription via its interaction with a DNA-binding protein Ume6 [11]. During vegetative growth Ume6 binds to promoters of early-meiosis genes and represses their expression. This requires association of Ume6 with the histone deacetylase Sin3/Rpd3 complex and chromatin remodeling complex Isw2 [12,13]. Absence of glucose and nitrogen results in the replacement of Sin3/Rpd3 and Isw2 by Ime1 and this switch activates the transcription of early-meiosis genes [14]. Rim11, the homolog of human Glycogen synthase kinase (GSK-3) phosphorylates Ime1 which enhances its interaction with Ume6 and thereby activates expression of early-meiosis genes [15]. Glucose also prevents transcription of *IME1* by inhibiting a kinase called Rim15 which promotes interaction of Ime1 with Ume6 [16]. Amongst various targets of Ime1 are two crucial genes *IME2* and *NDT80* which encode a Cdk-related kinase and a transcription factor for middle-meiosis genes, respectively.

G1-S transition

Entry into pre-mitotic S-phase requires the activity of Cdk bound to cyclins Cln1 and Cln2 which target the Clb-Cdk inhibitor Sic1 for degradation. During entry into pre-meiotic S-phase, Sic1 degradation is promoted by Ime2 instead of Cdk-Cln1/2 [17] . Mechanisms driving entry into pre-mitotic and pre-meiotic S-phases might be different as conditions that trigger entry into the two phases are contrastingly different (presence vs. absence of nutrients). In addition, the Cln-Cdk's inhibit *IME1* expression making entries into mitosis and meiosis mutually exclusive [18]. Cln3-Cdk induces expression of *CLN1* and *CLN2* during pre-mitotic G1 phase.

CLN3 expression is inhibited by Whi3 during meiosis which binds to *CLN3* mRNA and localizes it into discrete cytoplasmic foci [19].

Pre-meiotic S-phase

It is generally thought that DNA replication during mitotic and meiotic cell cycles require the same set of proteins. Consistent with this idea, the replication fork progression rates and choice of replication origins during pre-mitotic and pre-meiotic DNA replication are quite similar [20]. However the pre-meiotic S-phase is 2-3 times longer than pre-mitotic S-phase [21] and this is true in several organisms besides budding yeast. Preparation for recombination and homolog pairing could account for this difference. The length of the pre-meiotic S-phase is negatively regulated by the non-catalytic activity of Spo11 (the endonuclease that initiates recombination by introducing double-strand breaks on DNA) and positively regulated by the meiotic cohesin subunit Rec8 [22]. Clb5 and Clb6 cyclins are dispensable for pre-mitotic DNA replication but are essential for pre-meiotic DNA replication. While Clb1, Clb3 and Clb4 can compensate for the absence of Clb5 and Clb6 during vegetative growth [23], they cannot support pre-meiotic DNA replication for reasons unknown [24]. Pre-meiotic but not pre-mitotic DNA replication requires the protein Mum2 [25]. However the precise function of Mum2 in pre-meiotic DNA replication is unknown.

The four crucial innovations during meiosis

Following pre-mitotic DNA replication sister chromatids are bi-oriented on the mitotic spindle and segregated towards opposite poles during anaphase. However the fate of chromosomes after pre-meiotic S-phase is quite different. Four key differences between mitosis and meiosis I allow cells to halve the chromosome number. They are

a) Reciprocal recombination between homologous chromosomes leads to formation of crossovers or chiasmata which is essential for bi-orientation of recombined homologs or bivalents on the metaphase I spindle.

b) While sister kinetochores bi-orient during mitosis, they mono-orient during meiosis I i.e. they bind to microtubules emanating from the same spindle pole. This is essential for setting up the reductional mode of meiosis I chromosome segregation.

c) Cohesin at the centromeric regions is protected from cleavage by separase. Destruction of arm cohesion resolves chiasmata that link homologs together. Centromeric cohesion that persists after meiosis I is required for bi-orientation of sister centromeres on the meiosis II spindle.

d) S-phase is suppressed between meiosis I and II.

The crux of understanding how meiotic cells halve ploidy lies in deciphering how the above 4 processes occur.

I Recombination and formation of chiasmata

After pre-meiotic DNA replication the sister chromatids are connected by cohesin but there is no linkage between homologous chromosomes. The result of meiotic recombination is creation of a link between the homologs so that they can be pulled towards opposite spindle poles during meiosis I. Although recombination contributes to genetic diversity, it is important to remember that its principal function is to facilitate chromosome segregation during meiosis I. In the absence of recombination, homologous chromosomes are randomly segregated during meiosis [26,27].

Recombination in budding yeast is initiated by introduction of double-strand breaks (DSB's) on DNA by a topoisomerase- related protein Spo11 [26] with the help of several accessory proteins. Following this, a complex series of steps culminates in the formation of covalent connections between homologs called as crossovers (Figure 1). The DSB's are generated by a trans-esterification reaction and results in the covalent linkage of Spo11 to the 5' end of DNA at the DSB. The 5'ends linked to Spo11 are recognized by the RMX complex (Rad50/Mre11/Xrs2). RMX complex eliminates Spo11 from the 5' ends and in collaboration with Sae2/Com1 and Exo1, resects the 5' ends [28]. This results in the formation of single-stranded 3' overhangs which are bound by the bacterial RecA-related recombinase proteins Rad51 and Dmc1. The resulting ssDNA-Rad51/Dmc1 nucleoprotein filaments along with proteins Rad52 and Rad54 are recombinogenic and can scan the genome for homologous sequences. When the nucleoprotein filaments finds a match the ssDNA base-pairs with complementary strand from the homologous chromosome displacing the other strand to form a displacement loop (D-loop). Regulatory mechanisms ensure that homolog but not sister

chromatid is used to repair the double-strand break [29]. At this point there are 2 possibilities. The invading 3' strand could dissociate from its complementary strand and return to its original chromatid. This pathway referred to as synthesis-dependent strand annealing (SDSA) does not result in a covalent connection between the two homologs (non-crossovers). Alternatively, the displaced D-loop could base-pair with the second strand from the chromatid where the double-strand break was made. Extension of the 3' ends and ligation of the gaps results in a structure called as a double Holliday junction (dHJ). These dHJ's are then resolved by HJ resolvases Mus81/Mms4 and Yen1 which make a pair of nicks and ligations to create a crossover [30].

Formation of crossovers requires pairing of homologs and this is brought about by synapsis which involves assembly of a highly conserved proteinaceous structure called as synaptonemal complex (SC) that aligns homologs together. Recombination and SC assembly have to be tightly coordinated and this is achieved by the ZMM (an acronym for proteins Zip1, Msh4 and Msh5) group of proteins [31]. ZMM group comprise at least seven proteins Zip1-4 , Msh4, Msh5 and Mer3. The transverse filament protein Zip1 establishes stable homolog juxtaposition by polymerizing as an integral component of the SC. Zip2, Zip3, and Zip4 likely mediate protein-protein interactions, while Mer3, Msh4, and Msh5 directly promote steps in DNA recombination. Poorly understood mechanisms ensure that recombination generates at least one crossover between a pair of homologs which is essential for their bi-orientation on the meiosis I spindle. For a more detailed understanding of recombination, the readers are directed to a couple of reviews [31,32].

Linking pre-meiotic S-phase and DNA recombination

Multiple lines of evidence suggest that pre-meiotic DNA replication is coupled to recombination and homolog pairing. Firstly there was a direct correlation between the amount of replication that was observed in *clb5clb6* mutants and the number of double-strand breaks (DSB)'s they produced [33]. Secondly, deleting all replication origins on the left arm of chromosome III caused similar delays in replication and initiation of recombination in the same chromosomal region suggesting that replication is directly coupled to recombination [34]. The precise mechanism how this coupling is achieved is unknown. The presence of a replication fork is required for coupling replication to recombination as mutants that fail to

initiate replication (therefore will not produce replication forks) produce double-strand breaks normally and try to repair them [35]. Clb5-Cdk and Dbf4-dependent Cdc7 kinase are required for initiation of replication and for activating Mer2 (a meiosis-specific DSB protein) by phosphorylation [36,37]. By using the same set of proteins to activate initiation of pre-meiotic DNA replication and DSB formation cells might be able to co-ordinate the 2 processes.

Recombination checkpoint

The recombination checkpoint ensures that entry into meiosis I occurs after completion of DSB repair. Errors in DNA recombination and synapsis activate this checkpoint which causes an arrest in a stage of meiotic prophase called as pachytene. Pachytene is characterized by presence of unresolved double-Holliday junctions and persistent SC. Although Cdk activity is very low at this stage of meiosis, the term prophase is used for cytological reasons (due to appearance of condensed chromosomes with SC).

Recombination checkpoint requires kinases (Mec1 and Mek1), mitotic DNA damage checkpoint components (Rad17 and Rad24), SC components (Hop1 and Red1), Sir2 (a rDNA silencing protein), Pch2 (a AAA-based ATPase) and a histone methyl transferase (Dot1). For a detailed understanding of how this checkpoint senses errors in recombination please refer to a recent comprehensive review [38] . In this review we will only focus on how the checkpoint influences the meiotic cell cycle.

The recombination checkpoint primarily operates by controlling Cdk activity and expression of the transcription factor Ndt80. Cdk is regulated by phosphorylation and availability of its regulatory subunit Clb1. Errors in recombination activate Swe1 which inhibits Cdk by phosphorylating Cdc28 at Tyrosine-19 [39]. Expression of Clb1 is regulated by Ndt80 which is required for exit from pachytene [40]. Ndt80 binds to middle sporulation elements (MSE's) within the promoters of middle-sporulation genes (MSG's) and activates their transcription. In vegetative cells, the MSEs are bound by Sum1 which acts as a transcriptional repressor of MSGs [41]. Sum1 levels remain high during checkpoint activation and decline during exit from pachytene. Relative amounts of Sum1 and Ndt80 and their competitive binding to MSE determines whether cells exit from pachytene [42-44]. Cdk and Ime2 inactivate Sum1 by phosphorylating it and this leads to activation of Ndt80 [45]. The activity of Ndt80 might also be controlled by its nuclear localization [46] and phosphorylation by Ime2 [47].

Activation of Ndt80 results in the expression of more than 200 middle-meiosis genes which drive exit from pachytene. The middle-meiosis genes are also required for meiotic divisions and spore formation and include cyclins Clb1, Clb3 and Clb4, polo-like kinase Cdc5 and Ndt80 itself [48,49]. Activation of polo-like kinase Cdc5 results in the resolution of double-Holliday junctions and disassembly of SC [50]. Increased M-Cdk activity caused by expression of cyclins results in the separation of SPB's and formation of a metaphase I spindle.

II) Monopolar attachment of sister kinetochores

Homologs connected by chiasmata bi-orient on the meiosis I spindle. Tension created by sister chromatid cohesion distal to the chiasmata stabilizes the bi-oriented state. For homologs to go towards opposite spindle poles, it is essential that sister kinetochores are mono-oriented i.e. they bind to microtubules originating from the same spindle pole. This is in contrast to the scenario during mitosis where sister kinetochores bi-orient i.e. they bind to microtubules from opposite spindle poles (Figure 2A). So how is bi-orientation of sister kinetochores suppressed during meiosis I? Research over the last 10 years has shown that this is accomplished by the 'monopolin' complex which is composed of Csm1, Lrs4, Mam1 and Hrr25 [51-53]. Monopolin mutants attempt to bi-orient sister centromeres on the meiosis I spindle which results in a massive chromosome segregation defect.

Csm1 and Lrs4 are nucleolar proteins expressed during the mitotic cell cycle and are required for rDNA silencing and preventing unequal sister chromatid exchange at the rDNA repeats [54,55]. They interact with Tof2 which binds to rDNA via its interaction with the RENT (regulator of *n*ucleolar silencing and *t*elophase) complex composed of Net1, Cdc14 and Sir2. As cells exit from pachytene, Csm1 and Lrs4 are released from the nucleolus (Figure 2B) and this requires the activity of polo-like kinase Cdc5 [56,57]. Csm1 and Lrs4 associate with meiosis-specific protein Mam1 and casein kinase-1 Hrr25 to form the monopolin complex which associates with kinetochores. After nucleolar release Lrs4 is hyperphosphorylated by Dbf4-dependent kinase Cdc7 and Cdc5 in league with a meiosis-specific protein called Spo13. Lrs4 hyperphosphorylation is thought to assist monopolin binding to kinetochores [56-58]. The precise mechanism of how monopolin co-orients sister kinetochores is unknown. Crystal structure and electron microscopic analysis of Csm1/Lrs4 complexes indicates that they form

a V-shaped structure with 2 kinetochore binding globular domains separated by 10nm [59]. It was therefore suggested that monopolins could work by crosslinking two sister kinetochores via the 2 globular kinetochore-binding domains such that they face the same spindle pole. However the mechanism is likely to be more complex as the kinase activity of Hrr25 is required for monopolar attachment but not for monopolin binding to kinetochores [53]. Identifying substrates of Hrr25 at the kinetochores and monopolin binding sites on the kinetochore are crucial for understanding the mechanism of monopolar attachment.

It is noteworthy to point out that Pcs1 and Mde4 which are fission yeast homologs of Csm1 and Lrs4 respectively, are required to prevent merotelic attachments (where a single kinetochore binds to microtubules from both spindle poles) during mitosis and meiosis II but are not required for monopolar attachment during meiosis I [52,60,61]. An attractive hypothesis was proposed which might reconcile the contrasting phenotypes of monopolin mutants in budding and fission yeasts [52]. The fission yeast kinetochore has 3 MT binding sites compared to just 1 site for the budding yeast kinetochore. Monopolins were proposed to be nearest neighbour clamp for MT binding sites. In budding yeast monopolins clamp microtubule binding sites from 2 sister kinetochores. In fission yeast monopolins crosslink adjacent MT binding sites from the same sister kinetochore. Interestingly it has been recently reported that Pcs1 and Mde4 prevent merotelic attachment in fission yeast by targeting condensin complex (which is related to cohesin complex) to the kinetochores [62]. It is therefore possible that budding yeast monopolin complex targets condensin to the kinetochores to effect monopolar attachment during meiosis I. Consistent with this possibility, the condensin subunits Brn1 and Ycs4 are required for efficient monopolar attachment during meiosis I [63].

III) Protection of centromeric cohesion

During meiosis there are two nuclear divisions after one round of DNA replication. Since sister chromatid cohesion is established during DNA replication, complete loss of cohesion during meiosis I will result in separation of sister chromatids and random chromosome segregation during meiosis II. Meiotic cells have solved this problem by destroying cohesion in two steps. Cohesion along chromosome arms but not at centromeres is destroyed during meiosis I. Cohesion at centromeres is dissolved during anaphase II. Since separase becomes active

during anaphase I, centromeric cohesion has to be protected from separase cleavage. Protected centromeric cohesin is essential for bi-orientation of sister centromeres during meiosis II. Meiotic cells which fail to protect centromeric cohesion during meiosis I segregate dyad chromosomes randomly during meiosis II.

Protection of centromeric cohesion during meiosis I in budding yeast requires two crucial changes. Firstly the cohesin subunit Scc1 is replaced by the meiosis-specific Rec8. Meiotic cells expressing Scc1 instead of Rec8 fail to protect centromeric cohesion during anaphase I [51]. Secondly, meiotic cells target Sgo1 to the kinetochore to protect centromeric Rec8 from separase cleavage during meiosis I. Sgo1 belongs to a highly conserved family of coiled-coil proteins called as 'shugoshins' (guardian spirit in Japanese) and are required for protection of centromeric cohesion during meiosis I in budding yeast, fission yeast, flies, plants and mice [64-70].

How does Sgo1 protect centromeric cohesion? Work in the last 5 years has uncovered an elegant mechanism for protection of centromeric cohesion. Sgo1 recruits the protein phosphatase $PP2A^{Rts1}$ [71,72] to the kinetochores. The PP2A phosphatases consist of 3 subunits-a catalytic subunit (Pph21/Pph22), a scaffold subunit (Tpd3) and a regulatory subunit (Rts1/Cdc55). $PP2A^{Rts1}$ refers to a form that contains Pph21/22, Tpd3 and Rts1. Recruitment of $PP2A^{Rts1}$ to the kinetochores is achieved by its physical interaction with Sgo1 and a mutant form of Sgo1 that fails to bind to $PP2A^{Rts1}$ is defective in protection of centromeric cohesion [73]. $PP2A^{Rts1}$ opposes phosphorylation of meiotic cohesin subunit Rec8 at the centromeres by Hrr25, a casein kinase-1 and the Dbf4-dependent kinase Cdc7 [74]. This results in phosphorylated cohesin along the chromosome arms and dephosphorylated cohesin at the centromeres (Figure 3). It was already established that phosphorylation of mitotic cohesin subunit Scc1 by polo-like kinase Cdc5 sensitizes its cleavage by separase [75]. If a similar relationship between phosphorylation and sensitivity to separase extended to Rec8 then arm cohesin (phosphorylated) but not centromeric cohesin (dephosphorylated) would be susceptible to separase cleavage during meiosis I. Consistent with this idea, replacement of 24 phosphorylated serine/threonine residues in Rec8 by alanine (*rec8-24A*) inhibited its cleavage by separase [74]. Conversely phospho-mimetic substitution of 14 phosphorylated serine/threonine residues with aspartate (*rec8-14D*) caused loss of centromeric cohesion during meiosis I [74]. The above mechanism for protection of

centromeric cohesion is conserved in fission yeast in which it was shown that shugoshin-PP2A complex opposes casein kinase –1 dependent separase cleavage of Rec8 during meiosis I [76,77].

The polo-like kinase Cdc5 is required for phosphorylation of Rec8 and for its cleavage by separase during meiosis I [56,57]. However alanine -replacement of serine/threonine residues in Rec8 that were phosphorylated by Cdc5 had only a minor effect on kinetics of Rec8 cleavage during meiosis I [78]. Given the overwhelming evidence for casein kinase-1 and the Dbf4-dependent kinase, Cdc5 might play an indirect role in Rec8 phosphorylation. For instance, Cdc5 might be required for full activation of Cdc7 during meiosis. Consistent with this idea, Cdc5 physically interacts with Cdc7 during meiosis I [58].

IV) Exit from meiosis I

For halving ploidy, meiotic cells also have to prevent an occurrence of S-phase between meiosis I and II. Since high Cdk activity prevents licensing of replication origins [79], it is envisaged that meiotic cells might prevent a second round of DNA replication by maintaining relatively high levels of Cdk activity between meiosis I and II. Meiotic cells have to perform a careful balancing act in terms of Cdk activity during exit from meiosis I. The Cdk activity has to be reduced sufficiently to disassemble the meiosis I spindle but should be high enough to prevent licensing of replication origins. It is suggested that budding yeast cells have solved this tricky problem by employing the Cdc *F*ourteen *e*arly *a*naphase *r*elease (FEAR) network instead of *M*itotic *E*xit *N*etwork (MEN) to down regulate Cdk activity during exit from meiosis I [80,81].

Exit from mitosis requires inactivation of Clb-Cdk. This is achieved in budding yeast by the activation of the Cdk-antagonizing phosphatase Cdc14 during anaphase. Activated Cdc14 dephosphorylates Cdk substrates and targets the mitotic cyclins for degradation. Both FEAR and MEN signaling cascades activate Cdc14 during anaphase. However MEN but not FEAR is essential for mitotic exit as it causes a more complete inactivation of Cdk activity during anaphase.

How does FEAR work? Cdc14 is kept in an inactive state bound to a nucleolar anchor protein Net1 for much of the cell cycle (Figure 4A). Phosphorylation of Net1 by Clb-Cdk causes

dissociation of Net1-Cdc14 complexes and Cdc14 release from the nucleolus during anaphase [82,83]. Premature release of Cdc14 from the nucleolus is prevented by the protein phosphatase $PP2A^{Cdc55}$ which opposes Net1 phosphorylation by Cdk until early anaphase (Figure 4B) [84,85]. Inhibition of $PP2A^{Cdc55}$ by separase (which triggers the metaphase-anaphase transition by destroying sister chromatid cohesion) results in Net1 phosphorylation and Cdc14 release from the nucleolus (Figure 4C). The precise mechanism how separase inhibits $PP2A^{Cdc55}$ is not known but it requires assistance from two fungal-specific proteins Zds1 and Zds2 [86] and the kinetochore protein Slk19. Interestingly the proteolytic activity of separase is not required for its FEAR function. In a parallel branch of the FEAR pathway a nucleolar protein Spo12 promotes Cdc14 release from the nucleolus during anaphase. Net1-Cdc14 interaction is promoted by the nucleolar protein Fob1. Activation of Spo12 by Cdk-mediated phosphorylation during anaphase weakens Net1-Cdc14 interaction by inhibiting Fob1 [87,88].

The FEAR network is required for exit from meiosis I.

The Cdc14 release initiated by the FEAR network is transient. This is possibly because the declining Cdk activity is unable to sustain Net1 phosphorylation thus leading to return of Cdc14 to the nucleolus. FEAR-induced Cdc14 release activates MEN, a G-protein mediated signaling cascade, which causes a complete release of Cdc14 from the nucleolus and is essential for mitotic exit. Although the transience of FEAR-induced Cdc14 release limits its ability to control mitotic exit, it is however appropriate for exit from meiosis I which requires an incomplete loss of Cdk activity. Indeed, the FEAR network has been shown to be essential for exit from meiosis I. Mutations in *SPO12*, *SLK19* and *ESP1* genes delay Cdc14 release from the nucleolus and disassembly of anaphase I spindles [80,81]. Although these mutant cells are arrested in anaphase I the meiosis II events go unperturbed and they perform two nuclear divisions on the same spindle resulting in the formation of dyads (2-spored asci). Thus FEAR is essential for coupling the chromosome segregation cycle to spindle assembly and disassembly during meiosis. Conversely, premature activation of FEAR during meiosis caused by loss of $PP2A^{Cdc55}$ activity results in a failure to assemble bipolar spindles and undergo nuclear divisions [89,90]. These results highlight the importance of FEAR and its timing of activation in regulating exit from meiosis I.

Is FEAR upregulated during meiosis I?

Although much of the knowledge about FEAR has come out of mitotic experiments it is quite likely to operate in the same way during meiosis I. However action of FEAR during meiosis I but not during mitosis achieves disassembly of anaphase spindles. MEN is inactive during meiosis I as a few essential MEN components like Cdc15 and Tem1 are not expressed during meiosis I [91]. Therefore the question that arises is how does FEAR cause disassembly of anaphase I spindles. One possibility is that meiotic cells have intrinsically low levels of Cdk activity compared to mitotic cells thus leading to increased sensitivity to FEAR. An alternative possibility is that FEAR is upregulated during meiosis to enable disassembly of anaphase I spindles. A strong candidate for this role is the Cdk-related kinase Ime2 which has 2 peaks of expression during meiosis [92]. The first peak is during early meiosis which would be consistent with its role in initiating pre-meiotic DNA replication. The second peak of expression coincides with meiosis I but its functional significance is yet to be determined. Although Ime2 has overlapping substrate specificity with Cdk, the Ime2 phosphorylation sites are resistant to dephosphorylation by Cdc14 [93]. Therefore Ime2 was proposed to limit the activity of Cdc14 during exit from meiosis I and prevent re-replication. Just as Ime2 and Cdk collaborate during exit from pachytene by phosphorylating Sum1, they could also phosphorylate Net1 during exit from meiosis I. Since Ime2 phosphosites in Net1 will be resistant to dephosphorylation by Cdc14, the Cdc14 release will be stronger than during mitotic FEAR activation and might suffice to disassemble anaphase I spindles. Consistent with this possibility, *ime2* mutants accentuate the phenotype of FEAR mutants [94] Identifying substrates of Ime2 during meiosis I is key to understanding how exit from meiosis I is regulated in budding yeast.

Meiosis II

After exit from meiosis I, the spindle pole bodies duplicate and separate to form two sets of bipolar spindles (metaphase II). While chromosome segregation during meiosis I is reductional, it is equational during meiosis II and similar to mitosis. Mechanisms supporting monopolar attachment of sister kinetochores and protection of centromeric cohesion would have to be disabled to accomplish this switch. Centromeric cohesion is required for bi-orientation of sister centromeres during metaphase II. Separase becomes active again during

anaphase II and cleaves centromeric cohesin triggering the metaphase II- anaphase II transition. Although the levels of Sgo1 decline after meiosis I, it is still bound to chromatin until anaphase II [67] . Since centromeric cohesin is no longer protected during meiosis II, this suggests that either Sgo1 bound to chromosomes after meiosis I is not functional or activated separase is able to cleave centromeric cohesin as a result of high enzyme/substrate ratio. It is completely unknown how monopolins are inactivated after meiosis I.

How do meiotic cells ensure that there is adequate Cdk activity for meiosis II? Clue to how this might be achieved came from an elegant study investigating the kinetics of cyclin expression during synchronous meiosis [95]. While Clb1 was expressed during meiosis I, Clb3 was expressed only during meiosis II. Since *CLB1* and *CLB3* are both transcribed at the same time following Ndt80 activation, it implied that the lag in Clb3 expression was due to a delay in *CLB3* mRNA translation. A 153 base sequence at the 5' UTR of *CLB3* mRNA was necessary for meiosis II- specific accumulation of Clb3. The same sequence was also sufficient to prevent protein accumulation during meiosis I. It is not known how 5'UTR of *CLB3* mRNA prevents translation during meiosis I. It might recruit a meiosis I-specific RNA-binding protein which either prevents its access to the translational machinery or delays export of *CLB3* RNA from the nucleus to the cytoplasm. By engineering a delay in translation of *CLB3* mRNA meiotic cells might ensure that Cdk activity builds up quickly after meiosis I.

Following meiosis II, a forespore membrane (FSM) is synthesized within the cytoplasm of the mother cell which eventually becomes the plasma membrane of ascospores. The growth of FSM begins at the outer plaque of the SPBs and then proceeds outwards, encapsulating the 4 haploid nuclei.

Features of meiotic cell cycle regulation

A study of budding yeast meiosis has helped us to uncover general mechanisms to halve ploidy. Firstly the transcription of genes that are specifically required during meiosis is linked to nutrient starvation. Secondly, meiotic cells make subtle improvisations to the mitotic cell cycle machinery to effect dramatic changes that result in halving of ploidy. The use of meiosis-specific cohesin complex subunit Rec8 is an outstanding example. Rec8 is required for recombination, synaptonemal complex assembly and protection of centromeric cohesion. Thirdly, as in many processes in biology the use of phosphorylation and dephosphorylation as

a molecular switch is also true for the meiotic program in yeast. Fourthly meiotic cells use master kinases to regulate multiple key processes during meiosis. The Cdk-related Ime2 kinase is required for DNA replication and exit from pachytene. The Dbf4-dependent kinase Cdc7 is required for initiating DNA replication during the mitotic cell cycle. However during meiosis Cdc7 is also required for recombination, monopolar attachment of sister kinetochores and sensitizing arm cohesin cleavage during meiosis I. The casein kinase-1 is required for monopolar attachment of sister kinetochores and for sensitizing cohesin cleavage along chromosome arms during meiosis I. Cdc5 is required for chiasmata formation, monopolar attachment and for cohesin cleavage during meiosis I. By having master kinases, cells might be able to better coordinate the relative timing of landmark events during meiosis.

Are meiotic regulatory mechanisms conserved from budding yeast to humans?

Since meiosis is fundamental for all sexually reproducing organisms one would imagine the ploidy-halving mechanisms to be evolutionarily conserved. While mechanisms controlling recombination and possibly protection of centromeric cohesion are conserved, those regulating monopolar attachment and exit from meiosis I may have diverged significantly.

Multiple elements of the recombination pathway that leads to crossover between homologs are conserved from yeast to humans. Paralogs of genes encoding Spo11 (endonuclease that creates DSB's), Rad50 –Mre11-Xrs2 complex (processing of DSB's), Dmc1 (recombinase), Mus81-Mms4 and Yen1 (DHJ resolvases) are present in mammalian genomes. Inactivation of some of the above genes produce phenotypes expected from their deduced function in yeast [96]. Defects in recombination and synapsis also trigger an arrest in pachytene in mice suggesting the existence of a recombination checkpoint. However none of the genes required for this checkpoint have been identified. Interestingly the murine homolog of the yeast checkpoint protein Pch2 is not required for checkpoint signalling in mice but for recombination [97].

Members of the shugoshin family of proteins are required for protection of centromeric cohesion during meiosis I in mice. Inactivation of murine Sgo2 by RNAi in oocytes results in loss of protein phosphatase PP2A-B56 (counterpart of budding yeast $PP2A^{Rts1}$) binding to centromeres and premature sister chromatid separation during anaphase I [70]. Since separase is required for cleavage of arm cohesin during anaphase I in murine oocytes [98], it

is quite likely that Sgo2 protects cohesin from separase cleavage by recruiting PP2A-B56 during meiosis I.

Since homologs of Lrs4 and Csm1 are found only amongst closely related yeast and the fact that fission yeast counterpart (Pcs1/Mde4 complex) is not required for monopolar attachment, the mechanism of monopolar attachment in mammals is bound to be different. In fission yeast, cohesion at the core centromeric region mediated by cohesin containing meiosis-specific subunit Rec8 is required for monopolar attachment [99,100]. By juxtaposing the sister kinetochores side-by-side, centromeric cohesin constrains the sister kinetochores to face the same spindle pole during meiosis I [101]. Rec8's function in monopolar attachment appears to be conserved as inactivation of Rec8 results in bi-orientation of sister kinetochores during meiosis I in plants [102,103]. It remains to be tested whether Rec8 is also required for monopolar attachment during mammalian meiosis I.

Mechanism regulating exit from meiosis I in mammals is likely to be quite different from that in budding yeast. Although homologues of Cdc14 exist in humans, they do not appear to be required for cell cycle progression [104]. In addition, the orthologues of FEAR components in fission yeast are not required for nucleolar release of Cdc14 homolog Clp1 [105]. In fission yeast a meiosis-specific protein Mes1 inhibits APC which results in a partial destruction of cyclin (Cdc13) during exit from meiosis I [106]. This ensures that there is adequate Cdk activity for meiosis II. Inactivation of Mes1 results in complete destruction of cyclins and a failure to undergo meiosis II. In *Xenopus* oocytes, the presence of a meiosis-specific S-Phase inhibitor Mos and absence of Cdk-inactivating Wee1 kinase are important for preventing an extra round of DNA replication between the 2 divisions [107,108]. How mammalian cells exit from meiosis I is not known.

Conclusion and future perspective

Although most of the genes regulating the key meiotic events in budding yeast have been identified, the precise molecular mechanisms by which the encoded proteins work remain poorly understood. Structural biochemistry and in vitro reconstitution of physiological reactions with purified protein complexes will be crucial for advancing our knowledge of meiosis. For a complete understanding of recombination one would require to reconstitute each step in the proposed pathway with defined components. Poor synchrony of meiotic

yeast cultures may have limited interpretation of mutant phenotypes. Live imaging of meiosis and synchronous meiotic cultures are two powerful tools that will play an increasingly important role in analyzing the phenotypes of mutants. The discovery that translation of *CLB3* mRNA occurred during meiosis II was possible because of a new protocol for synchronous meiosis. Since phosphorylation is used as a molecular switch in multiple meiotic processes, quantitative phospho-proteomics of synchronous meiotic cultures might be illuminating. Ultimately it is crucial to test whether the mechanisms discovered in yeast are conserved in mammals. A more detailed understanding of mammalian meiosis might shed light on basis of infertility and aneuploidy-related disorders in humans.

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Figure legends

Figure 1 **Pathway for meiotic recombination in budding yeast**

Recombination is initiated by creation of double-strands breaks on DNA by Spo11. The 5'ends are resected by the Rad50-Mre11-Xrs2 (RMX) complex in league with Com2/Sae1 and Exo1. Nucleoprotein filaments formed by association of recombinases Rad51 and meiosis-specific Dmc1 with single-stranded DNA scan the genome for homologous sequences. When a successful match is found the 3' end base pairs with the complementary strand displacing the other strand forming a D-loop. At this stage there are two possible fates. In the synthesis-dependent strand annealing (SDSA) pathway the invading strand dissociates from the homologous strand and re-anneals with its complementary strand leading to a non-crossover. A crossover is formed when the displaced strand base-pairs with the strand complementary to the invading strand to form a double-Holliday junction which is subsequently resolved a pair of nick and ligation reactions by resolvases Mus81/Mms4 and Yen1.

Figure 2 **Monopolar attachment of sister kinetochores during meiosis I**

A) Cohesion between sister chromatids (in blue) is mediated by cohesin (yellow rings). A specialized structure called as the kinetochore is formed at the centromere on each chromatid. The kinetochore is composed of 3 distinct layers the inner (black), the central (red) and the outer (gray) kinetochores. Sister kinetochores bind to microtubules (green rods) emanating from opposite spindle poles during mitosis (bi-orientation).

B) Csm1 and Lrs4 proteins localize to the nucleolus via their binding to Tof2 which is bound to rDNA via its association with the RENT (Regulator of nucleolar silencing and telophase) complex . After exit from pachytene Cdc5 triggers release of Csm1 and Lrs4 from the

nucleolus. Csm1/Lrs4 complex associates with meiosis-specific Mam1 and casein kinase-1(Hrr25) subunits to form the monopolin complex. After nucleolar release, Lrs4 is phosphorylated by Dbf4-dependent kinase Cdc7 and Cdc5 in league with meiosis-specific protein Spo13. Monopolin complex associates with sister kinetochores and cross-links them such that they bind to microtubules from the same spindle pole. For the sake of simplicity chiasmata are not shown in the figure.

Figure 3 **Protection of centromeric cohesion during meiosis I**

A) During metaphase I Sgo1 targets the Protein Phosphatase $PP2A^{Rts1}$ to the kinetochore which shields centromeric cohesin from phosphorylation by casein kinase -1 (Hrr25) and Dbf4-dependent kinase (DDK). Separase cleaves arm cohesin (phosphorylated) to resolve chiasmata during anaphase I but does not cleave centromeric cohesin (dephosphorylated).

B) Loss of Sgo1/$PP2A^{Rts1}$ function after meiosis I results in phosphorylation of centromeric cohesin. Separase gets reactivated during meiosis II and cleaves centromeric cohesin to separate dyad chromosomes.

Figure 4 The FEAR network regulates exit from meiosis I

A) Until prophase I, Cdc14 is bound to the nucleolus via its physical interaction with its competitive inhibitor Net1 which is enhanced by the replication fork barrier protein Fob1.

B) Increase in Cdk activity following exit from pachytene promotes phosphorylation of Net1 but this is opposed by the protein phosphatase $PP2A^{Cdc55}$ restraining Cdc14 release from the nucleolus. This state persists until metaphase I.

C) During anaphase I , separase is activated by destruction of its inhibitor securin which is targeted for proteasomal degradation by APC^{Cdc20} . Activated separase in association with Slk19 inhibits $PP2A^{Cdc55}$ via Zds1/2 proteins which results in phosphorylation of Net1 and Cdc14 release from the nucleolus. In addition, activation of Spo12 by Cdk-mediated phosphorylation weakens the effect of Fob1 in stabilizing Net1-Cdc14 interaction. The Cdc14

released antagonizes Cdk activity which is sufficient to cause spindle disassembly but not licensing of DNA replication origins.

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Figure 1

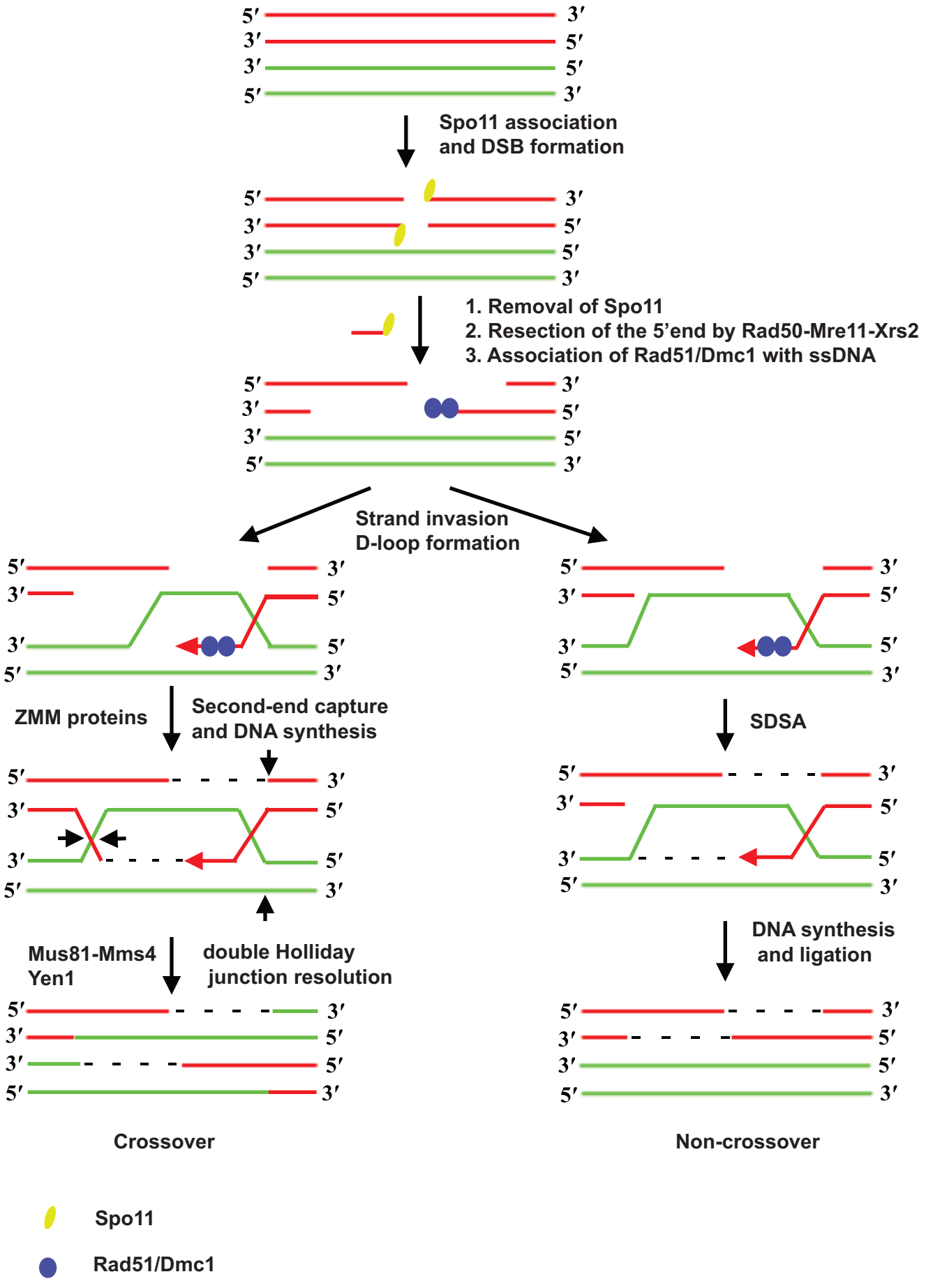

Figure 1

Figure 2

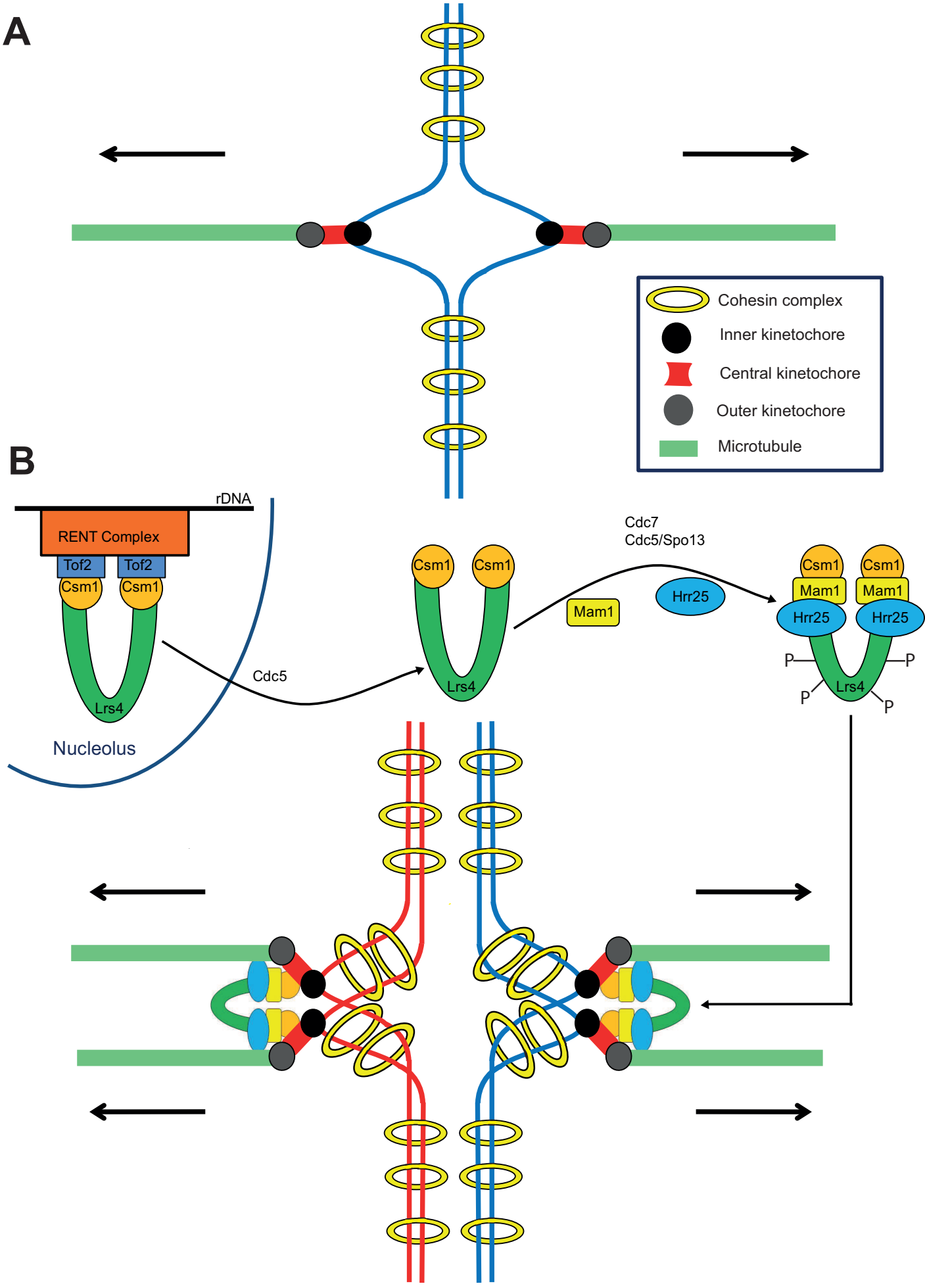

Figure 2

Figure 3

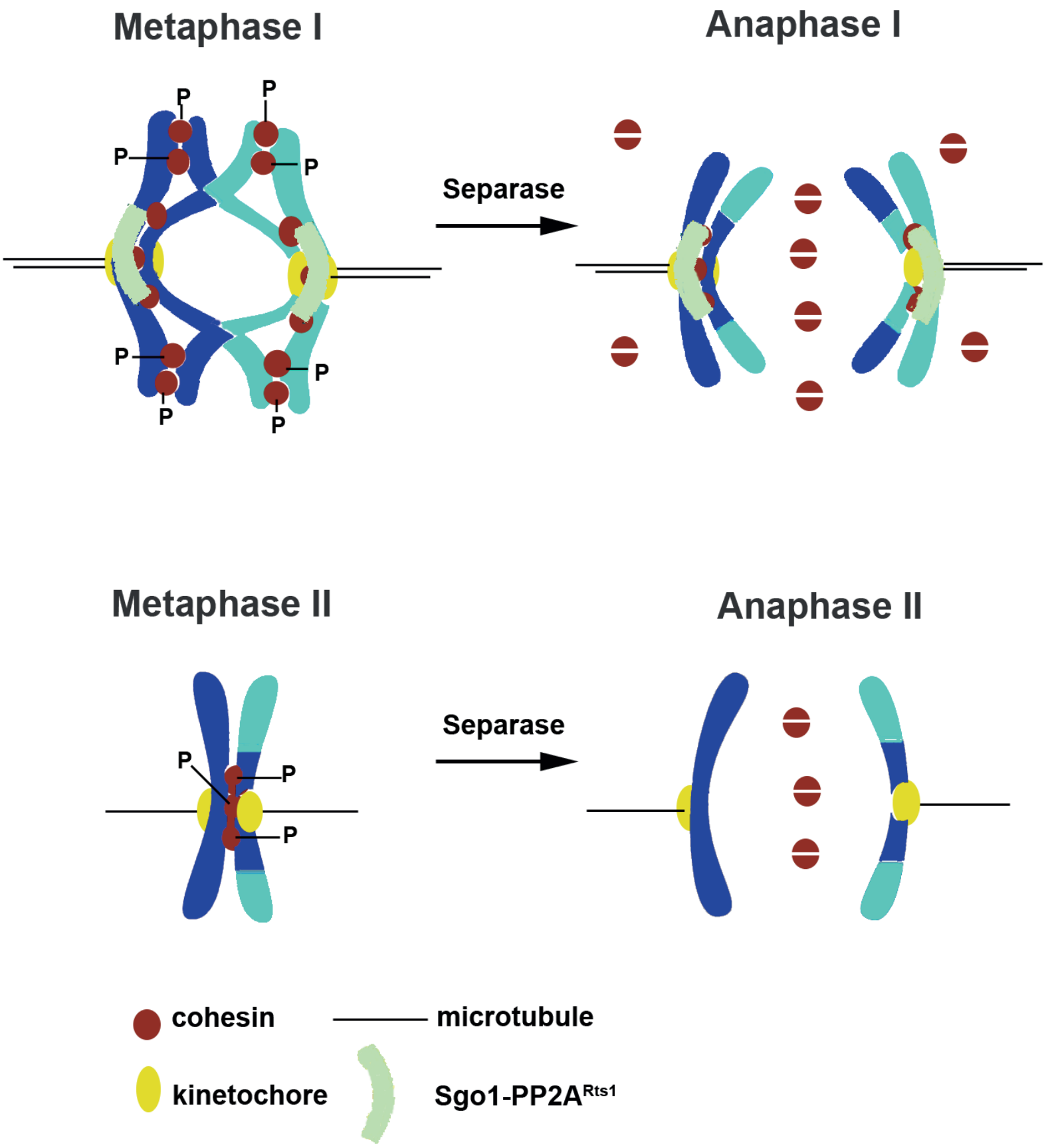

Figure 3

Figure 4

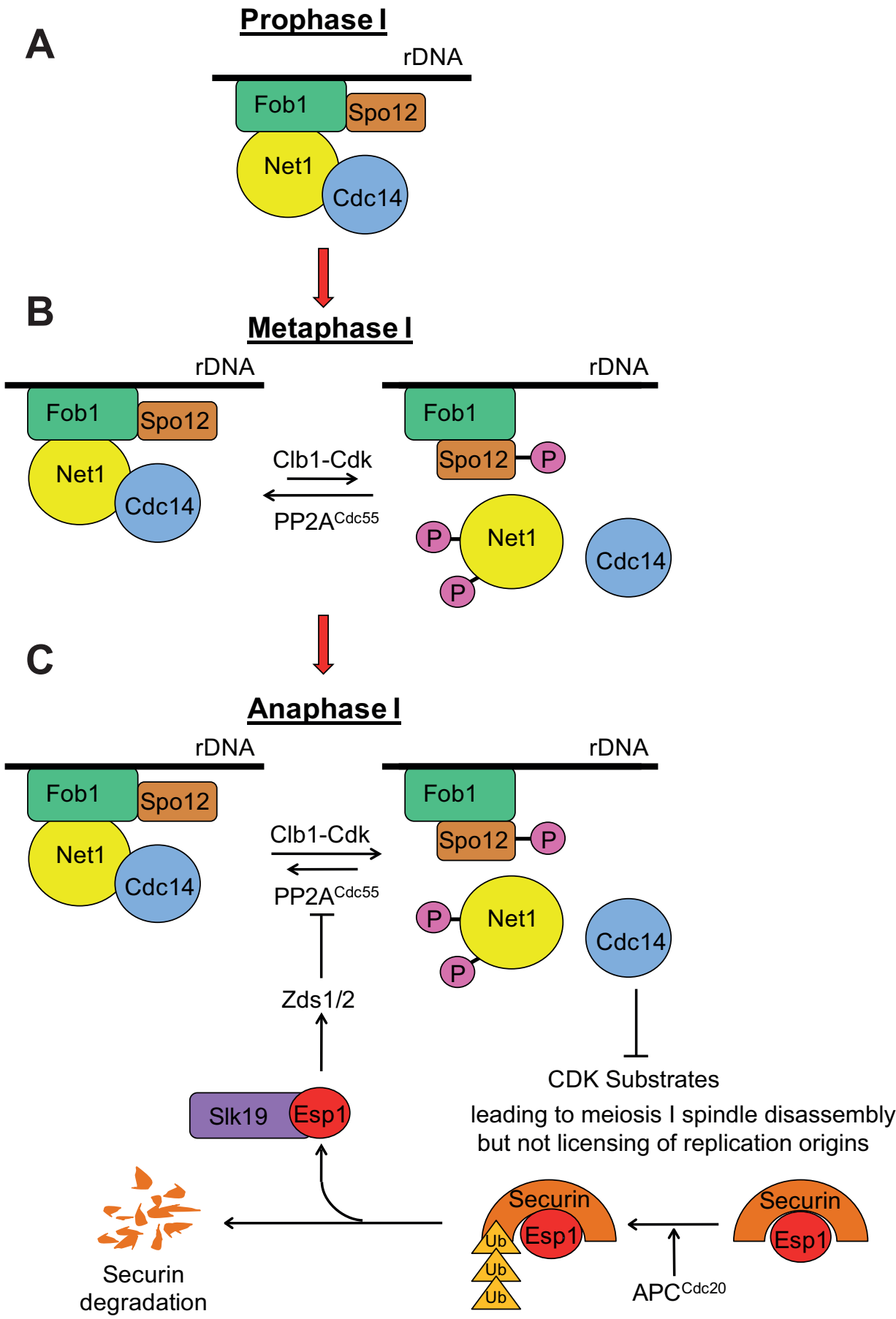

Figure 4